S. pombe cdc11p, together with sid4p, provides an anchor for septation initiation network proteins on the spindle pole body

Andrea Krapp, Susanne Schmidt*, Elena Cano and Viesturs Simanis

Background: The signal for the onset of septum formation in the fission yeast *Schizosaccharomyces pombe* is transduced by the septation initiation network (SIN). Many of the components of the SIN are located on the spindle pole body during mitosis, from where it is presumed that the signal for septum formation is delivered. *Cdc11* mutants are defective in SIN signaling, but the role of *cdc11* in the pathway has remained enigmatic.

Results: We have cloned the *cdc11* gene by a combination of chromosome walking and transfection of cosmids into a *cdc11* mutant. Cdc11p most closely resembles *Saccharomyces cerevisiae* Nud1p and is essential for septum formation. Cdc11p is a phosphoprotein, which becomes hyperphosphorylated during anaphase. It localizes to the spindle pole body at all stages of the cell cycle, in a sid4p-dependent manner, and cdc11p is required for the localization of all the known SIN components, except sid4p, to the SPB. Cdc11p and sid4p can be coimmunoprecipitated from cell extracts. Finally, like its *S. cerevisiae* ortholog Nud1p, cdc11p is involved in the proper organization of astral microtubules during mitosis.

Conclusions: We propose that cdc11p acts as a bridge between sid4p and the other SIN proteins, mediating their association with the spindle pole body.

Address: Cell Cycle Control Laboratory, Swiss Institute for Experimental Cancer Research (ISREC), 1066 Epalinges, Switzerland.
Present address: *CRBM - CNRS UPR 1086, 1919 Route de Mende, 34293 Montpellier Cedex 5, France.

Correspondence: Viesturs Simanis
E-mail: viesturs.simanis@isrec.unil.ch

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Background

The fission yeast *Schizosaccharomyces pombe* has proven to be an excellent model organism for studying cytokinesis. It grows by tip elongation and divides by the formation of a medially placed division septum. The position of the division site is defined at the onset of mitosis, probably by signals emanating from the nucleus, and results in the assembly of an actomyosin-based contractile ring at the cell cortex [1]. Septum formation is initiated at the end of anaphase, when contraction of the actomyosin ring is thought to guide synthesis of the division septum. The onset of septation in fission yeast is signaled through the septation initiation network (SIN) reviewed in [2]. SIN mutants assemble apparently normal contractile rings but fail to initiate ring constriction and septum formation. Nuclear division and growth continue in the absence of cell division, leading to the production of elongated, multinucleated cells [3, 4]. Activation of the SIN requires the polo-like kinase *plo1* [5]. Signaling is mediated through the GTPase spg1p, which is negatively regulated by the byr4p-cdc16p GAP, and interacts with the protein kinase cdc7p at the onset of anaphase. Two other protein kinases, sid1p and sid2p, and their associated proteins (cdc14p and mob1p, respectively) are thought to act downstream of spg1p. These proteins are all located on the spindle pole body (SPB) during mitosis, and the sid2p-mob1p protein kinase also associates with the medial ring during septation.

Localization of the protein kinases cdc7p, sid1p, and sid2p to the SPB requires spg1p [6–8]. Mob1p is required for the localization of sid2p [9, 10], and cdc14 is required for the localization of sid1p and mob1p/sid2p [6]. Sid4p is required for the SPB localization of spg1p, byr4, and cdc7 [11, 12], suggesting that it may provide an anchor for SIN proteins on the SPB. However, no interaction between sid4p and any SIN component has been demonstrated to date [12].

Cdc11 mutants have been identified in many screens for mutants defective in cytokinesis and cell cycle progression [3, 4, 13, 14]. Two lines of reasoning have led to the suggestion that *cdc11* acts at an early stage in SIN signaling. First, overexpression of either *spg1* or *cdc7* can rescue *cdc11-136* [15, 16]. Second, cdc7p, sid1p, cdc14p [6], and sid2p [8] do not localize to the SPB in *cdc11-123* at 36°C, and the localization of mob1p [10] and byr4p [11] are both impaired. Neither sid2p nor mob1p associate with the medial ring in *cdc11-123* [8, 10]. In contrast, sid4p is still associated with the SPB in a *cdc11-123* mutant [12].

We have cloned the *cdc11* gene and shown that is essential for septum formation. Cdc11p localizes to the SPB throughout the cell cycle, dependent upon sid4p, but not upon other components of the SIN. Conversely, spindle pole body localization of SIN proteins, with the exception of sid4p, depends on cdc11p. We show that cdc11p interacts with sid4p, suggesting that they provide an anchor for the SIN on the spindle pole body.

Results

Cloning of *S. pombe cdc11*

Attempts to clone *cdc11* by rescue of *cdc11* mutants using plasmid libraries yielded only multicopy suppressors ([17] and unpublished data [D. Poloni, S.S., L. Cerutti, and V.S.]). Genetic mapping experiments showed that *cdc11* is on the lower arm of chromosome III [18]. The physical position of the *cdc11* gene was defined by integrating fragments of cosmids into the genome together with a marker, followed by crosses to *cdc11-136* to determine the genetic distance to the integration site. Cosmids covering the *cdc11* gene were cotransfected into *cdc11-136* together with a nonreplicating marker plasmid, and complementation was tested by replicating to 36°C. Only SPCC1739 rescued consistently, and subcloning identified the ORF responsible for the rescue as SPCC1739.11c. Three tests indicated that this was the *cdc11* gene. First, SPCC1739.11 is adjacent to *ppe1*. A cross of *cdc11-136* to *ppe1::ura4*$^+$ (covered by a ppe1+ plasmid to permit mating) gave 64PD, 1TT, and 0 NPD, indicating very close linkage between the two genes. Second, SPCC1739.11 cloned on a nonreplicating vector carrying the *ura4*$^+$ gene was integrated into *cdc11-136 ura4-D18* cells. This strain was crossed to *ura4-D18*, and free spore analysis yielded 973 cdc$^+$ura$^+$ colonies, 1015 cdc$^+$ura$^-$ colonies, 1 cdc$^-$ura$^-$ colony, and 4 ura$^+$cdc$^-$ colonies, indicating very close linkage of the integrated *ura4* gene to the *cdc11* mutation. Third, sequencing of SPCC1739.11 revealed the presence of mutations in two *cdc11* alleles (see below).

Database searches indicate that cdc11p is most closely related to *S. cerevisiae* Nud1p, a spindle pole body component involved in astral microtubule organization and mitotic exit [19–21]. The two proteins are most related in their C-terminal region, which contains the multiple leucine-rich repeats (see Figure S1 in the Supplementary material available with this article online).

cdc11 is an essential gene

A *cdc11* null allele was generated by replacing part of the coding region with the *ura4*$^+$ gene in a diploid background. Meiotic products of this strain produced only ura$^-$ colonies, indicating that *cdc11* encodes an essential protein. The *cdc11::ura4*$^+$ spores germinated, but did not form septa, becoming elongated and multinucleated (Figure 1a). Germinating *cdc11::ura4*$^+$ spores formed medial rings at mitosis, and F-actin patches were present at the tips in interphase (Figure 1a). Furthermore, staining for cdc15p [22] and cdc4p [23] showed the expected ring formation in mitotic cells (Figure 1b), arguing that the block to septum formation in *cdc11::ura4*$^+$ cells occurs after actomyosin ring formation.

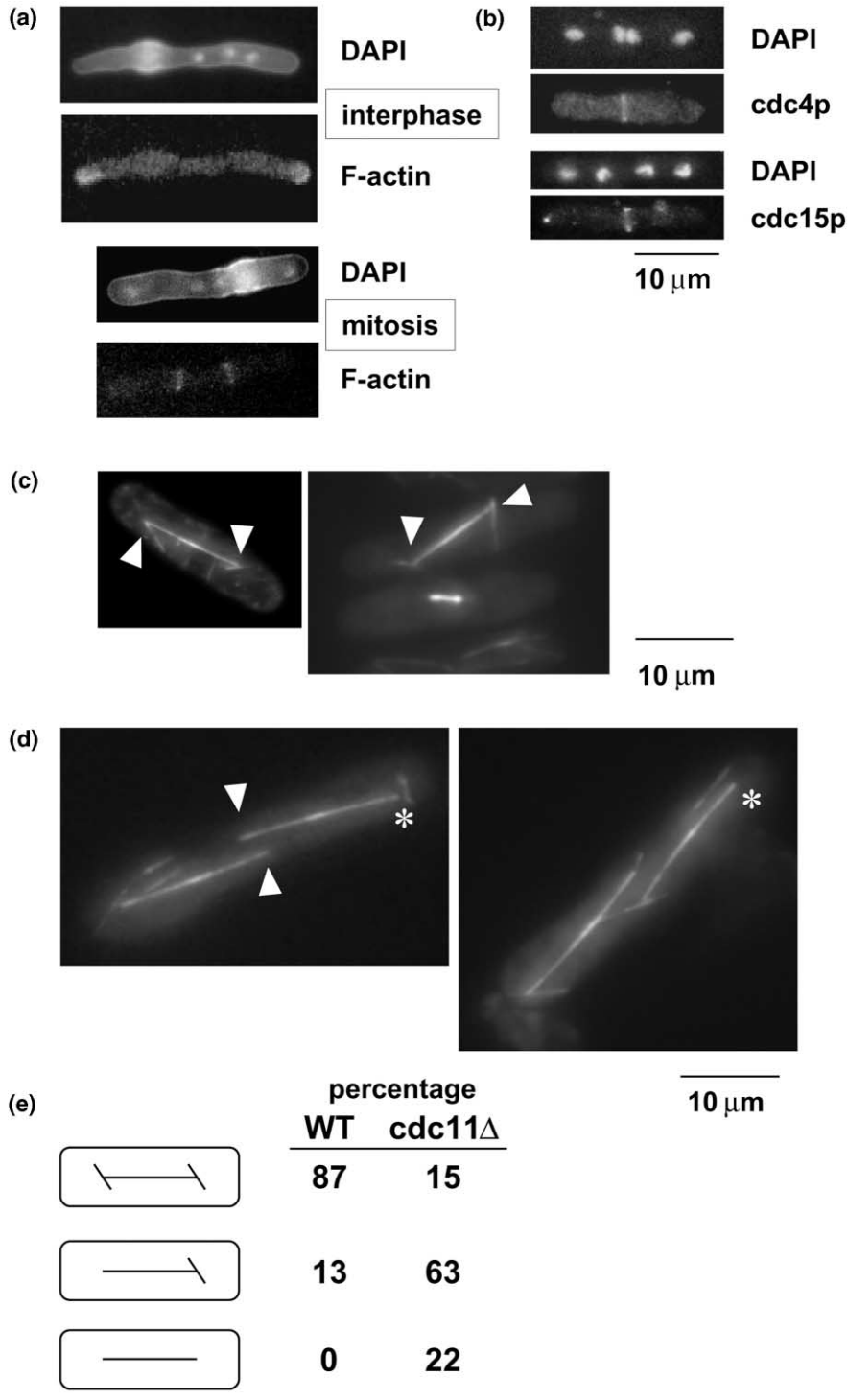
Multicopy *spg1* or *cdc7* can rescue the *cdc11-136* mutant at 36°C [15, 16]. We therefore tested whether increased expression of any of the SIN genes could rescue the *cdc11::ura4*$^+$ allele. The diploid heterozygous for *cdc11::ura4*$^+$ was transformed to leucine prototrophy with pREP-family plasmids expressing SIN genes from the *nmt1* promoter. Colonies were allowed to sporulate, and spores were plated on medium selective for both uracil (the null allele) and leucine (the REP plasmid). No haploid colonies were obtained, whether expression of the SIN gene was induced or not. Thus, overproduction of none of the currently known SIN genes can bypass the requirement for cdc11p. Conversely, increased expression of *cdc11* from the full-strength *nmt1* promoter, induced or uninduced, did not rescue any of the other SIN mutants (data not shown). Induction of *cdc11* expression from the full-strength *nmt1* promoter in wild-type cells had little effect upon cell cycle progression, though occasional elongated, binucleate cells were observed (<1% of the population).

Nud1p, the *S. cerevisiae* homolog of cdc11p, is involved in organizing astral microtubules during S, G2, and M [20]. We therefore examined whether astral microtubule arrays were normal in *cdc11::ura4*$^+$ cells. In wild-type cells, the astral microtubules emanate from both spindle pole bodies (Figure 1c; also see [24, 25]). A diploid heterozygous for the *cdc11* null mutant was transformed to leucine prototrophy with a plasmid expressing GFP-α-tubulin [26], and the structure of the microtubules was examined in germinating *ura4*$^+$ spores. It was found that, in late anaphase cells, astral microtubule arrays were frequently absent, or detached from, one or both spindle pole bodies (Figure 1d). Quantitation showed that, while 87% of mitotic spindles in wild-type cells had astral microtubules attached to both spindle pole bodies, in *cdc11::ura4*$^+$, only 15% of mitotic spindles displayed bipolar astral microtubules (Figure 1e). Thus, like *S. cerevisiae* Nud1p, cdc11p also plays a role in astral microtubule organization during mitosis.

Cdc11p is hyperphosphorylated during mitosis and localizes to the SPB throughout the cell cycle

To study cdc11p, the protein was tagged at its C terminus by the addition of either three copies of the 12CA5 (HA) epitope or by GFP. In all of the experiments described, the tagged copy of the gene replaces the wild-type copy of *cdc11*. *Cdc25-22 cdc11-HA* cells were synchronized by

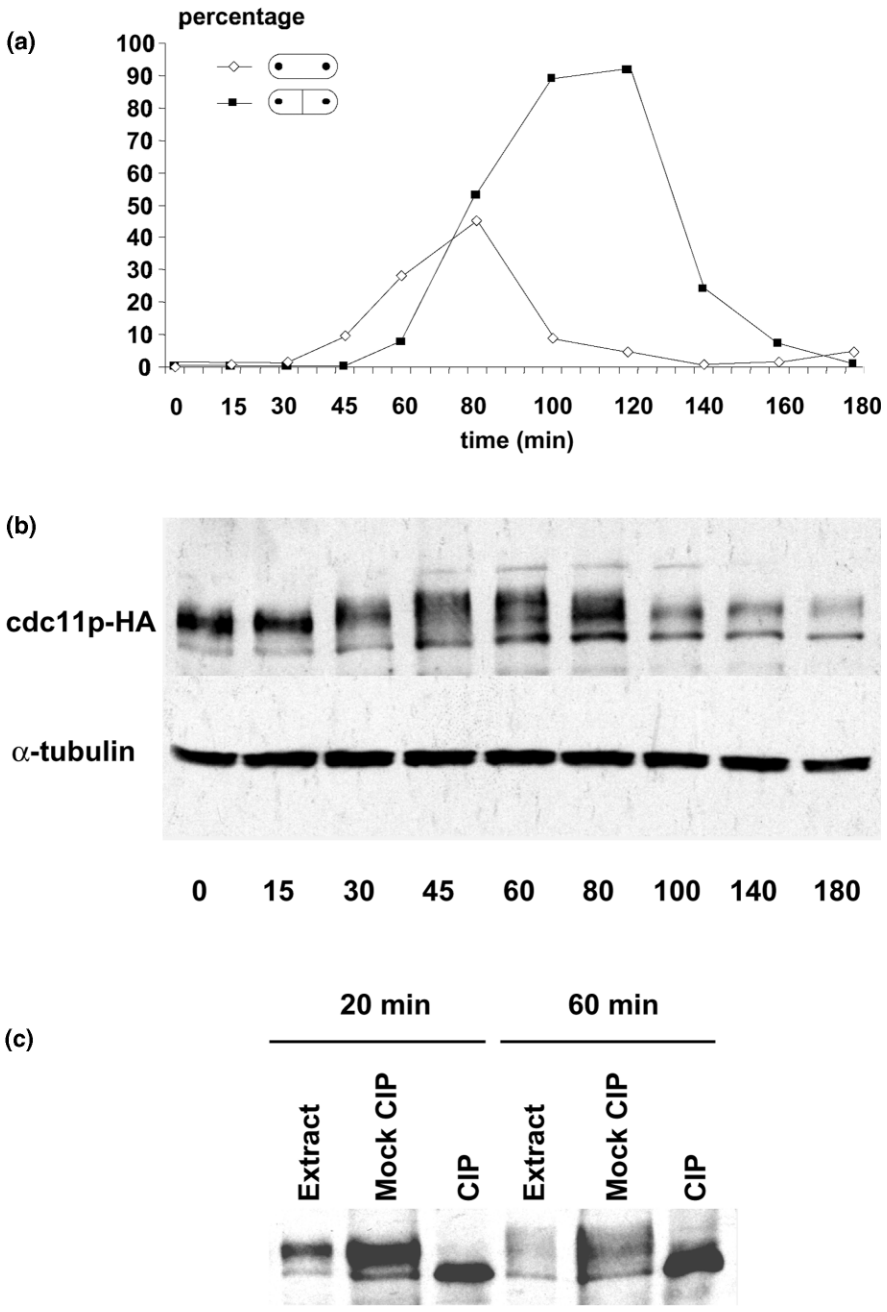
Figure 1



Characterization of the phenotype of *cdc11::ura4*$^+$ cells. A diploid heterozygous for *cdc11::ura4*$^+$ was sporulated, and spores were inoculated into medium lacking uracil. Cells were fixed after approximately 20 hr of growth, and cells were stained with the indicated reagents. **(a)** F-actin staining of cells with rhodamine-conjugated phalloidin. The upper panels show an interphase cell, and the lower panels show a mitotic cell. Note the presence of F-actin at the tips in the interphase cell and the F-actin rings in the mitotic cell. The broad band in the DAPI-calcofluor staining is the residue of the germinating spore. **(b)** Staining for medial ring components. The cell in the upper panels was stained with rabbit antiserum to cdc4p. The cell in the lower panels was stained with rabbit antiserum to cdc15p. **(c)** Wild-type cells were transformed with a plasmid expressing GFP-α-tubulin, and living mitotic cells were examined. Triangles indicate the position of astral microtubule arrays. **(d)** The diploid was transformed with a plasmid expressing GFP-α-tubulin before sporulation. Spores were prepared and germinated, selecting for the plasmid and the *cdc11* null allele. The asterisks indicate spindle poles, where the astral microtubule array has detached from the pole. In the left panel, the triangles indicate spindle poles lacking astral microtubule arrays. **(e)** Quantitation of astral microtubule defects during mitosis. Mitotic wild-type and *cdc11::ura4*$^+$ spores bearing the GFP-α-tubulin plasmid were examined, and the percentage of cells with astral microtubules attached to both, one, or neither spindle pole was determined. A total of 50 wild-type and 100 *cdc11::ura4*$^+$ cells were examined.

arrest-release (Figure 2a). Western blotting showed that cdc11p-HA was present throughout the cell cycle, with a slight decrease in its level as cells completed cytokinesis (Figure 2b). Moreover, cdc11p-HA, which already migrated as multiple forms during G2, was further modified upon entry into mitosis, returning to the faster-migrating forms as cells underwent cytokinesis. Following treatment of protein extracts with alkaline phosphatase (CIP), the multiple forms of cdc11p-HA collapsed to a single band, corresponding to the fastest-migrating species seen in G2 cells (Figure 2c). These results indicate that cdc11p is a phosphoprotein that is hyperphosphorylated from the

Figure 2



cdc11p is hyperphosphorylated during mitosis. *cdc11-HA cdc25-22* cells were synchronized by arrest-release, and samples were removed at intervals. **(a)** A plot of the percentage of binucleated and septated cells after release from the arrest. **(b)** A Western blot of protein extracts prepared at the indicated time points, blotted with 12CA5 (top panel) and TAT-1 (bottom panel). **(c)** Extracts from the indicated time points were treated with calf-intestinal alkaline phosphatase in the presence (Mock CIP) or absence (CIP) of phosphatase inhibitors, and they were then analyzed by Western blotting with 12CA5.

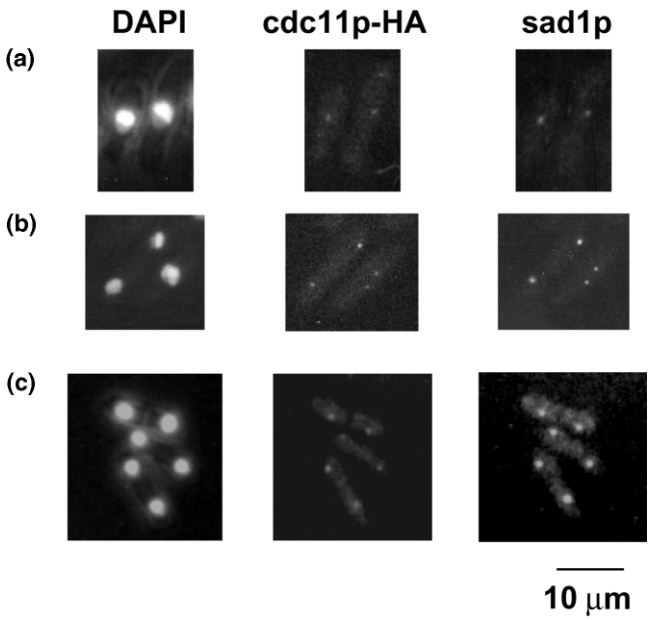
beginning of anaphase until septation. Analysis of total RNA extracted from cells synchronized by elutriation showed that the steady-state level of *cdc11* mRNA did not change throughout the cell cycle (data not shown).

Indirect immunofluorescence of fixed *cdc11-HA* cells revealed the presence of dots at the periphery of the nuclei (Figure 3). These dots coincide with those visualized by staining for sad1 (Figure 3), a component of the SPB [27]. A cdc11p signal was detected on the spindle pole body at all stages of the cell cycle, including the two poles of the mitotic spindle both early (Figure 3b) and late (Figure 3c) in mitosis. We noticed that the intensity of the signal increases during anaphase and is lowest in early interphase cells. Similar data were obtained by observation of the GFP fluorescence in living *cdc11-GFP* cells (data not shown).

SPB localization of cdc11p requires sid4p, but none of the other SIN proteins

To determine whether the localization of cdc11p depends on other proteins known to be required for septum forma-

Figure 3



cdc11p is associated with the spindle pole body. *cdc11-HA* cells were fixed and stained with DAPI, 12CA5 (cdc11-HA), and rabbit antiserum to the spindle pole body component sad1p. **(a)** Interphase cells, **(b)** early (right-hand cell) and late (left-hand cell) mitotic cells, and **(c)** septating (top cell) and two late mitotic cells.

tion, cdc11p-HA or cdc11p-GFP localization was examined in various thermosensitive SIN and F-actin ring formation mutants (Figure 4). cdc11p-HA or cdc11-GFP localized to SPBs in all of the mutants tested, with the exception of the *sid4-SA1* mutant, in which cdc11p-HA was no longer detectable. Western blotting indicated that cdc11p-HA levels are unchanged in *sid4-SA1* at the non-permissive temperature (data not shown). Cdc11p was also detected on the spindle pole body at all cell cycle stages in the germinating spores of *cdc7* and *spg1* null mutants, eliminating the possibility that the presence of the protein at the spindle pole body in these mutants at 36°C was due to the use of a hypomorphic heat-sensitive allele (data not shown). It has been proposed that *plo1* acts upstream of the SIN [5]. However, cdc11p was also on the spindle pole body in the septation-defective mutant *plo1-4*, indicating that its localization does not depend upon *plo1* activity.

SPB localization of the SIN proteins, with the exception of sid4p, requires cdc11p

Previous studies of the mutant *cdc11-123* showed that the localization of some SIN components requires functional cdc11p. However, mutant alleles of *cdc11* differ in their genetic interactions with other SIN components, suggesting that some may be hypomorphs [28]. Therefore, we examined the localization of SIN proteins and other septation regulators in germinating *cdc11::ura4*$^+$ spores. Indirect immunofluorescence demonstrated that both sid4p and the SPB marker sad1p [27] still localize to the SPB (Figure 5a,b). The protein kinase plo1p, which is thought to function upstream of the SIN [5], was also located on the SPB (Figure 5c), as described previously in wild-type cells [29, 30]. Localization of clp1p/flp1p [31, 32] was also normal (not shown). In contrast, spg1p-GFP, sid2p-GFP, cdc7p-GFP, mob1p-GFP, and byr4p were all absent from the SPB (data not shown). These results suggest that the block to septum formation in *cdc11::ura4*$^+$ cells is due to a failure to recruit (and/or retain) SIN components, with the exception of sid4p and plo1p, to the SPB.

Localization of mutant cdc11p and SIN components in *cdc11* mutants

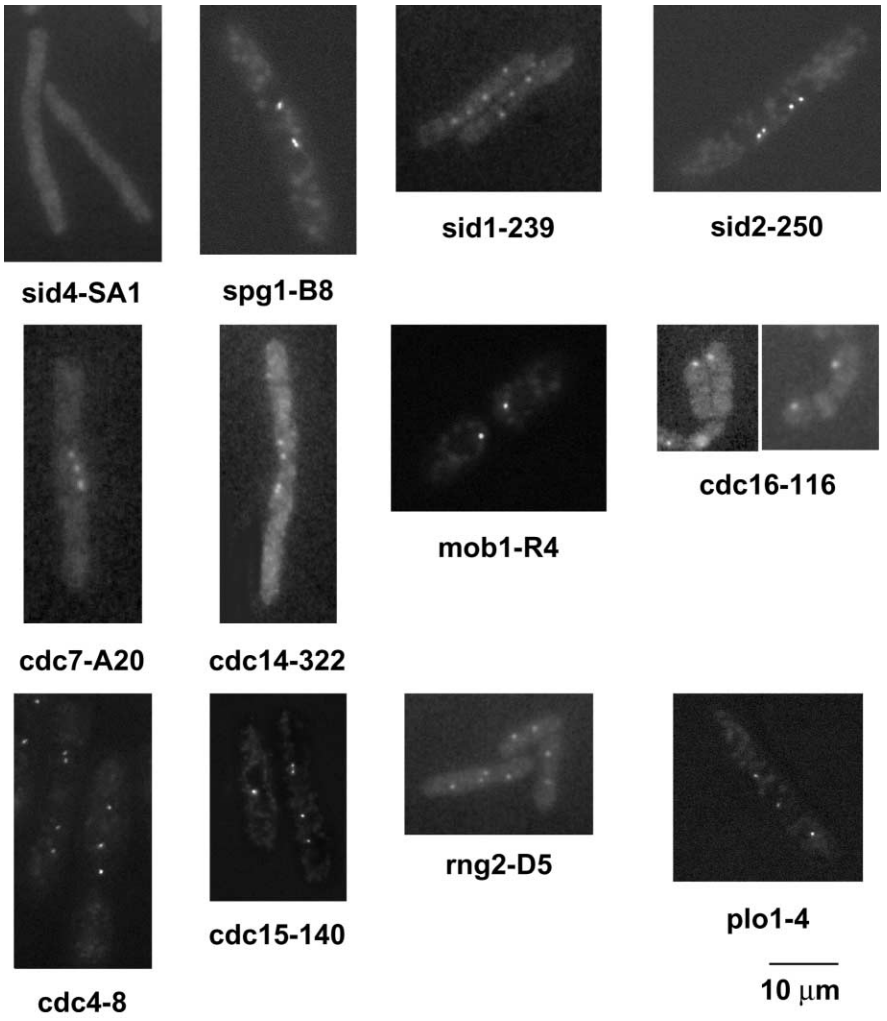
To determine whether mutant forms of cdc11p can associate with the SPB, GFP was fused to the C terminus of the mutant cdc11p in *cdc11-136* and *cdc11-123*. At 25°C, both cdc11-123p-GFP and cdc11-136p-GFP were located on the spindle pole body (Figure 6a,b). However, at 36°C, the SPB signal of cdc11-123p-GFP was either undetectable or very faint (Figure 6c), while cdc11-136p-GFP was readily detectable on the spindle pole body (Figure 6d). Western blotting indicated that the level of cdc11-123p-GFP and cdc11-136p-GFP did not change significantly upon the shift from 25°C to 36°C (data not shown). These results suggest that septation defects in *cdc11-123* result from a failure of mutated cdc11p to localize properly to the SPB.

Since cdc11-136p-GFP still associates with the spindle pole body at the restrictive temperature, we investigated whether other SIN components localized normally in this background after growth at 36°C for 5 hr. The results are shown in Table S1 in the Supplementary material available with this article online. cdc7p, sid1p, or byr4p were not detected at the spindle pole body in *cdc11-136* at 36°C. In contrast, both spg1p and sid2p were present on the spindle pole bodies at 36°C. We were unable to preserve sid2p rings after fixation at 36°C in wild-type cells and were therefore unable to determine whether sid2p was present at the medial ring in *cdc11-136* (data not shown). Previous studies had indicated that mob1p localization to the SPB during mitosis was unaffected in *cdc11-136*, though the medial ring association could not be assessed [9]. These findings contrast in some respects with previous observations in the *cdc11-123* background at 36°C, where sid2p-GFP did not localize to either the SPB or the medial ring and the signals for mob1p were faint or absent [6, 8, 11].

Cdc11p interacts with sid4p

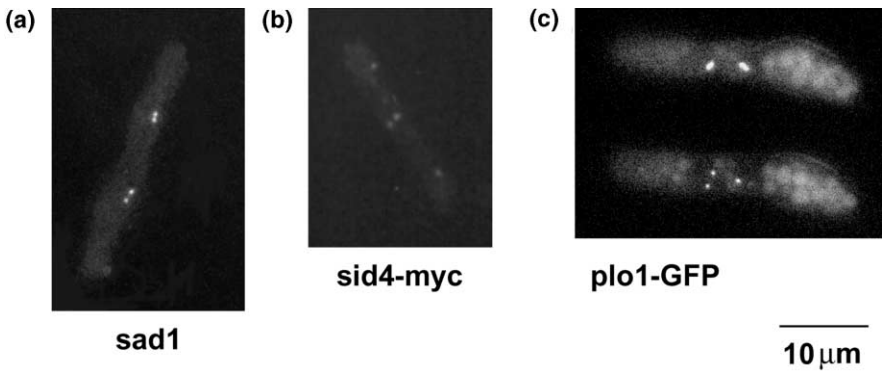
The fact that sid4p can associate with the SPB independently of cdc11p, while cdc11p requires sid4p to localize

Figure 4



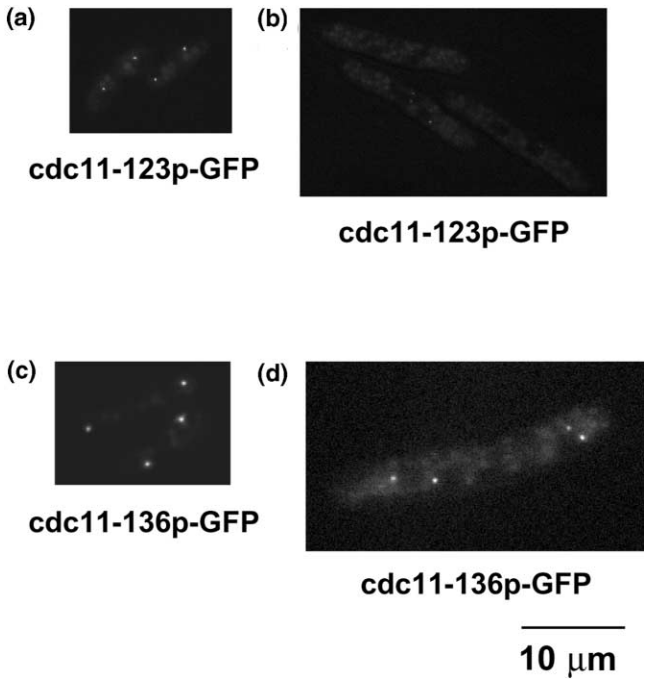
Localization of cdc11p to the spindle pole body depends on sid4p. The *cdc11-HA* allele was crossed into the indicated genetic background. Cells were grown to midexponential phase, at 25°C, shifted to 36°C for 5 hr, fixed, and stained with 12CA5. Note that a spindle pole body signal is present in all of the mutants except *sid4-SA1*. The *cdc11-HA* allele was used for detection in the *cdc16, sid4, cdc14, sid1, cdc7, and rng2* backgrounds, while the *cdc11-GFP* allele was used in the *spg1*, *sid2*, *mob1*, *cdc4*, *cdc15*, and *plo1* mutants.

Figure 5



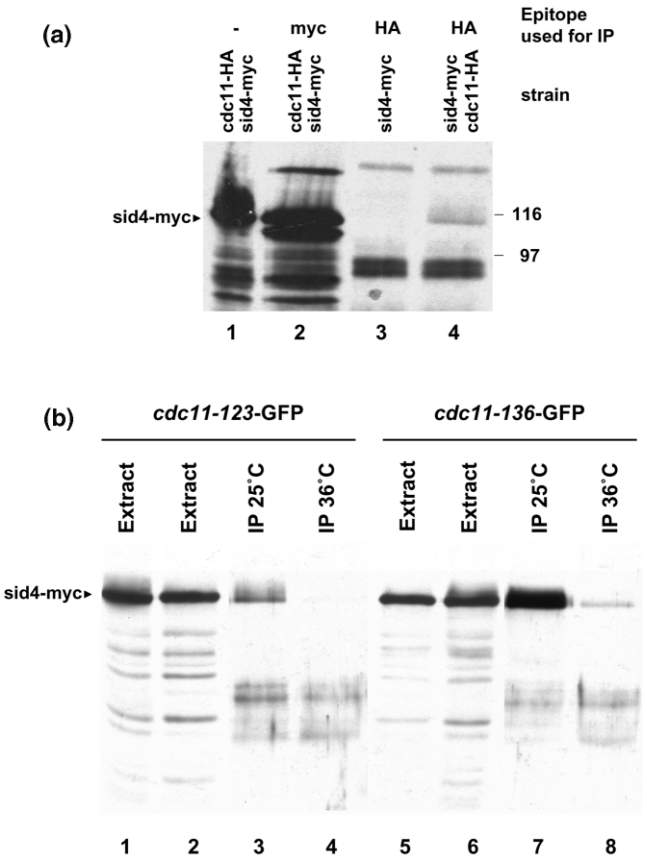
Localization of SIN proteins in *cdc11::ura4*$^+$ cells. **(a)** Germinating *cdc11::ura4*$^+$ cells were fixed and stained with rabbit antiserum to sad1p. Note the presence of multiple dots, corresponding to the spindle pole bodies. **(b–f)** A haploid strain carrying the *cdc11::ura4*$^+$ allele covered by a plasmid was crossed to strains carrying the indicated markers, and spores were prepared. Plasmid inheritance during meiosis is inefficient, so most spores do not inherit the *cdc11* plasmid. Spores were inoculated into medium selecting for the *cdc11* null allele and the tagged gene, and the localization of the tagged protein was examined in elongated, multinucleated cells as described in the Materials and methods. (b) Cells were fixed and stained with the monoclonal antibody 9E10. (c) GFP fluorescence was observed in living cells.

Figure 6



Localization of mutant cdc11p proteins. **(a–d)** The indicated strains were grown at 25°C ([a] and [c]), or shifted to 36°C for 4 hr ([b] and [d]), and cells were fixed and stained with anti-GFP antibodies.

Figure 7



Sid4p and cdc11p interact. **(a)** Protein extracts were prepared from either *cdc11-HA sid4-myc* or *cdc11*$^{+}$ *sid4-myc* strains, and immunoprecipitates were made using either 12CA5 or 9E10. Immunoprecipitates were analyzed by Western blotting and probed with 9E10. Lane 1: protein extract from the *cdc11-HA sid4-myc* strain. Lane 2: immunoprecipitation with 9E10, from the *cdc11-HA sid4-myc* strain. Lane 3: immunoprecipitation with 12CA5 from a *sid4-myc* strain. Lane 4: immunoprecipitation with 12CA5 from a *sid4-myc cdc11-HA* strain. **(b)** Protein extracts were prepared from either *cdc11-123-GFP sid4-myc* or *cdc11-136-GFP sid4-myc* strains, either at 25°C (lanes 1, 3, 5, and 7) or after a 5-hr incubation at 36°C (lanes 2, 4, 6, and 8). Total extracts (lanes 1, 2, 5, and 6) and immunoprecipitates prepared using rabbit anti-GFP (lanes 3, 4, 7, and 8) were Western blotted and probed with 9E10.

to the spindle pole body, led us to test whether sid4p interacts with cdc11p. Native protein extracts were prepared from cells containing single, differently tagged copies of both cdc11p and sid4p, expressed under the control of their own promoters. Immunoprecipitates using 12CA5 from a cdc11p-HA sid4p-myc strain contained sid4p-myc (Figure 7a). Immunoprecipitation with 9E10 (anti-myc), followed by Western blotting with 12CA5, indicated the presence of cdc11p in sid4p immunoprecipitates (data not shown). Thus, cdc11p and sid4p can be precipitated as a complex from cell extracts.

Since cdc11-123p-GFP does not localize correctly to the spindle pole body at 36°C, while cdc11-136p-GFP does, we examined whether the interaction of sid4p with cdc11p is perturbed in these mutants. Strains expressing sid4p-myc and either cdc11-123p-GFP or cdc11-136p-GFP were incubated at 36°C for 5 hr, and native protein extracts were prepared before and after the shift. The amount of sid4p-myc extracted was unaffected by the shift from 25°C to 36°C (Figure 7b; lanes 1, 2, 5, and 6). Immunoprecipitation of the mutant cdc11p using antiserum to GFP coimmunoprecipitated sid4p-myc from extracts of cells incubated at 25°C in both *cdc11-136* and *cdc11-123* (Figure 7b, lanes 3 and 6). When immunoprecipitates were performed using extracts prepared from cells incubated at 36°C, little or no sid4p-myc was coimmunoprecipitated with cdc11-123p-GFP (Figure 7b, lane 4). In contrast, sid4p-myc was still coimmunoprecipitated with cdc11-136p-GFP (Figure 7b, lane 8), though at reduced levels. Western blotting indicated that equal amounts of cdc11-123p-GFP and cdc11-136p-GFP were immunoprecipitated at 25°C and 36°C (data not shown).

To examine which region of cdc11p was required for interaction with sid4p, we performed a two-hybrid analysis. Full-length cdc11p was able to interact with sid4p (Table 1). N- and C-terminal truncations of cdc11p demonstrated that the C-terminal half of the protein is impor-

Table 1

Two-hybrid interactions between *sid4p* and *cdc11p*.

Bait	Prey	β-galactosidase activity
None	sid4	960
Cdc11	none	8
Cdc11	sid4	1625
Cdc11-N	none	7
Cdc11-C	none	10
Cdc11-N	sid4	1235
Cdc11-C	sid4	6830

Cdc11 is full-length *cdc11*. *sid4* is full-length *sid4*, described previously [12]. Cdc11-N is amino acids 1–578. Cdc11-C is amino acids 442–1046. β-galactosidase activity is normalized to the number of cells in the assay.

tant for interaction with sid4p in this assay (Table 1). Interestingly, sid4p interacts more strongly with the C-terminal half of cdc11p than the full-length protein. It is possible that the N terminus of cdc11p interferes with the interaction, perhaps reflecting a negative regulatory role for this domain.

The *cdc11* ORF was amplified by PCR from *cdc11-123* and *cdc11-136*. Sequence analysis revealed the presence of a single mutation in *cdc11-136* that changes N768 to M, while two mutations were found in *cdc11-123*: R947 to H and T1041 to I. These mutations lie within the C-terminal region of cdc11p that interacts with sid4p in the two-hybrid assay (Table 1).

Discussion

Role of cdc11 in the SIN

We have shown that the localization of cdc11p to the SPB depends on sid4p and that the two proteins interact. Moreover, the localization of all SIN components, with the exception of sid4p, depends upon cdc11p for stable association with the spindle pole body. We therefore suggest that sid4p is the SPB-anchoring protein for cdc11p and that together they facilitate loading of SIN proteins to the spindle pole body.

S. cerevisiae Nud1p interacts with the Bub2p-Byr4p complex, which is the GAP for Tem1p (the ortholog of spg1p) [20]. To date, we have failed to detect any interaction between cdc11p and either cdc16p (the counterpart of Bub2p) or byr4p, by either coimmunoprecipitation or in two-hybrid analysis. Nor have we been able to detect any interaction of cdc11p with either spg1p or cdc7p. It is possible that, in fission yeast, these complexes may exist only transiently or that they are labile and dissociate during extraction and the subsequent processing steps. Alternatively, bridging between cdc11p and the downstream SIN might be mediated by an as yet unidentified SIN protein.

Increased expression of some SIN components can advance or delay the onset of septum formation [15, 16, 33–35]. However, strong overexpression of *cdc11* had no significant effect upon septation, as demonstrated previously for its binding partner, sid4p [12]. This result could be explained if cdc11p needs to associate with the spindle pole body to be able to interact with other SIN components, perhaps as a result of being modified in some way after binding to the SPB. In this case, an excess of unmodified cdc11p in the cytoplasm would have little effect.

Analysis of both the *cdc11-123* mutant [6, 8] and the *cdc11* null allele (this work) demonstrate that all of the SIN proteins, except sid4p, require cdc11p to localize to the spindle pole body. Since the cdc11-123p does not localize correctly to the SPB or form a complex with sid4p, we conclude that *cdc11-123* phenocopies the *cdc11* null mutant with regard to SIN protein localization. In contrast, only a subset of SIN proteins fail to localize in *cdc11-136*. Spg1p, mob1p, and sid2p are present on the spindle pole body, while cdc7p and sid1p are not. It is noteworthy that the *cdc11-136* mutant can be rescued by a small increase in expression of cdc7p [15], while the *cdc11-123* mutant cannot (data not shown). The mutation in *cdc11-136* may identify a domain that is important for the loading and/or maintenance of cdc7p at the spindle pole body. Future studies will attempt to identify proteins interacting with this region of cdc11p.

What is the role of phosphorylation of cdc11p?

cdc11p is a phosphoprotein and becomes hyperphosphorylated at the onset of anaphase, suggesting that phosphorylation of cdc11p plays a regulatory role in SIN signaling. Phosphorylation of cdc11p at anaphase coincides with cdc7p association with the spindle pole body. Since many components of the SIN are distributed asymmetrically on the two poles of the mitotic spindle later in anaphase [6, 7, 11, 36], it is tempting to speculate that changes in the phosphorylation state of cdc11p on one pole before the other might play a role in mediating this event.

Which kinase(s) phosphorylate(s) cdc11p? One candidate could be the protein kinase plo1p, since it is thought to act upstream of the SIN and is activated and spindle pole body associated from the earliest times in mitosis [5, 30]. Alternatively, cdc2p could be involved in the phosphorylation of cdc11p at the onset of mitosis, given the presence of multiple cdc2p consensus sites in the N-terminal half of the protein and the timing of its activation. Finally, one or more of the downstream SIN kinases could phosphorylate cdc11p as part of a feedback loop. Studies of the *S. cerevisiae* MEN provide a precedent for such events [37–39]. Future studies will attempt to establish which of these kinases phosphorylates cdc11p.

What is the role of astral microtubules in septation?
Though the phenotypes of the *cdc11* and *nud1* mutants are different, both proteins are required for the formation of a normal astral microtubule array. Nud1p binds the γ-tubulin receptor Spc72p to the outer plaque of the spindle pole body and thereby helps anchor the γ-tubulin microtubule nucleating complexes to the SPB [20]. In the absence of cdc11p, astral microtubules are either absent or appear to detach from the spindle pole body. As expected, this is also the case in *sid4-SA1* (data not shown), where cdc11p does not localize to the spindle pole body. Our preliminary analysis of germinating *cdc11::ura4*$^+$ spores suggests that astral microtubules frequently form and then detach from the spindle pole bodies (A.K. and V.S., unpublished data).

The role of astral microtubules during the *S. pombe* mitotic cell cycle is unclear, but they could help to orient the spindle along the long axis of the cell [24, 40]. The *S. pombe* spindle orientation checkpoint may monitor attachment of astral microtubules to the region of contractile ring [40]. One attractive possibility is that the signal for ring contraction and septation is transmitted from the spindle pole bodies to the contractile ring via the astral microtubules. However, it is unlikely that this is the sole trigger for septation, since mutants in which microtubule formation is impaired (e.g., *nda3-KM311*) can still septate if cell cycle progression is permitted by the use of checkpoint mutants [41]. Nonetheless, it is interesting to note that, in *S. cerevisiae*, the interaction of microtubules with the bud neck is thought to be important for coordinating mitotic spindle position with mitotic exit [42].

Intriguingly, the astral array is frequently missing from one pole of the spindle, but not from the other, suggesting that the two spindle pole bodies in *cdc11::ura4*$^+$ may differ in their ability to nucleate and/or retain astral microtubules. These data are consistent with earlier observations showing that the SPBs behave differently during mitosis with respect to septation regulators and that SPB duplication may be conservative [6, 7, 11, 36, 43].

Conclusions

In this paper, we have presented the cloning and initial analysis of the *S. pombe cdc11* gene. Cdc11p is most closely related to *S. cerevisiae* Nud1p and is essential for septation. Null mutants become elongated and multinucleated, indicating that cdc11p is not required for either DNA synthesis or mitosis. F-actin rings are formed, and both cdc4p and cdc15p, two essential components required for ring assembly, are present. Thus, unlike its *S. cerevisiae* ortholog, which is required for mitotic exit [19, 20], *S. pombe cdc11* is essential for signaling the onset of septum formation. Cdc11p is required for the localization of all SIN proteins, except sid4p, to the spindle pole body. Cdc11p localization to the spindle pole body in turn depends upon sid4p. We suggest that cdc11p, together with sid4p, facilitates the loading of SIN proteins onto the spindle pole body.

Materials and methods

Yeast methods and strains
S. pombe strains were grown in yeast extract (YE) or minimal medium with appropriate supplements [44].

Cloning of cdc11
Cosmids originating from chromosome III from the region thought to contain *cdc11* (see Sanger center fission yeast database http://www.sanger.ac.uk/Projects/S_pombe/Chr3.shtml) were cotransfected with the nonreplicating vector pura4Δars into the strain *cdc11-136 ura4-D18*. This vector is derived from pura4 [44]. The Δars derivative was constructed by digestion with EcoRI and self-ligation, creating a nonreplicating vector that transforms cells to uracil prototrophy by integration. Since the recipient strain carries the *ura4-D18* deletion [45], stable transformation to uracil prototrophy can only be obtained by nonhomologous recombination or by recombination between the plasmid and cosmid, followed by integration. Approximately 2 μg vector and 10 μg cosmid were used per transfection. Transformants were selected for uracil prototrophy and then replica plated to YE plates containing phloxin B at 36°C. Each cosmid transfection was performed at least twice, in independent experiments. Only cosmid SPCC1739 reproducibly yielded more than 70% of the ura$^+$ colonies capable of growth at 36°C.

Supplementary material
Supplementary material including a list of strains used and methods for protein extraction, immunoprecipitation, and microscopy are described in the Supplementary material, available at http://images.cellpress.com/supmat/supmatin.htm.

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